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Discontinuity of the Basement Membrane in Fibrosing Basocellular Carcinomas and Basosquamous Carcinomas of the Skin: An Immunohistochemical Study with Human Laminin and Type IV Collagen Antibodies

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Thirteen basocellular carcinomas (BCC) of different histologic types and 5 basosquamous carcinomas (BSC) of the skin were stained for laminin and type IV collagen with rabbit antibodies against the human basement membrane (BM) proteins, using an immunoperoxidase technique. The BM around the tumor aggregates contained both laminin and type IV collagen, and was continuous and distinct in all the nonfibrosing BCCs but indistinct or interrupted in the fibrosing BCCs and BSCs. The BM was not influenced by the focal adnexal differentiation of the BCC cells. The disintegrity of the BM in the fibrosing BCCs and BSCs may reflect some kind of disturbance in the interaction between the neoplastic epithelium and the connective tissue stroma, and be connected with the more aggressive nature of these tumors compared with ordinary BCCs. Thus local aggressive behavior seems to be accompanied by defects in the BM.

Basocellular carcinomas (BCCs) of the skin differ with respect to their clinical and histologic picture and also their biologic behavior [1–4]. Most cases follow a benign clinical course, but some show a deep local invasion and a tendency for recurrences. A BCC seldom metastasizes. Reliable methods for distinguishing the aggressive tumors from the ordinary ones have so far been lacking [5], although it has been suggested that an infiltrative, morphea-like appearance of the BCC sheets, and rich sclerosing or hyalinizing connective tissue stroma around them, may predict aggressive behavior [1,3]. In view of this, the relation between the tumor epithelium and the connective tissue stroma has been intensively studied for many years [6–9], with special interest having been focused on the basement membrane (BM) between the epithelial and stromal components [9–11]. The BM around the BCC sheets has been shown to be intact and continuous in some electron microscope studies [12] but discontinuous in others [10,11], and defects in it have been revealed by immunohistochemical staining for bullous pemphigoid antigen [13–15], which is limited to the BM of the stratified squamous epithelia.

The chemical constituents common to all BMs have been extensively characterized in recent years [16,17]. One of these components is type IV collagen, which is organized into a netlike structure [18] with the individual molecules linked to each other at one end to form a particularly resistant structure known as the 7-S collagen domain of type IV collagen [18]. The noncollagenous components of BMs include a large glycoprotein known as laminin, a heparan sulfate containing proteoglycan, entactin, and at least in some BMs, fibronectin [16,17]. Type V collagen, although a pericellular component rather than a real structural constituent of the BMs, is often also found in the vicinity of these membranes [16,17]. Laminin, type IV collagen, and type V collagen recently have been identified in intact and continuous BMs around BCC aggregates using immunohistochemical staining [15,19,20].

The present study was undertaken to determine whether immunohistochemical staining for the BM components laminin and type IV collagen in BCCs and BSCs of the skin bears any relation to the differentiation of the epithelium or to stromal fibrosis.

MATERIALS AND METHODS

Eighteen biopsy samples of nonmetastatic cutaneous basal cell tumors fixed with 10% formaldehyde and embedded in paraffin were sectioned at 5 μm, stained with hematoxylin and eosin, and classified into differentiated and nondifferentiated BCCs and BSCs using the criteria of Lever and Schaumburg-Lever, Pinkus and Mehregan, and Borel [21–23] (Table I). A BCC was considered differentiated when tumor cell areas showing adnexoid differentiation, e.g., structures resembling hair follicles, sweat glands, or sebaceous glands, were found. A BCC composed of pure basal cells arranged in sheets, cords, and islands was classified as nondifferentiated. Basal cell tumors showing cells of intermediary differentiation between a basal cell and a squamous cell were classified as BSCs (see Fig 4). The tumors were further grouped into fibrosing and nonfibrosing types according to the growth type of the epithelium and the amount of connective tissue stroma. The fibrosing category included sclerosing, hyalinizing, and infiltrating tumor types [1,3,22]. The BMs were classified as follows: (1) continuous and distinct, when the BM was seen to envelope every tumor cell aggregate to form a continuous line of equal thickness and without disruptions, (2) continuous but indistinct, when the BM around some tumor islands was thin and seen only with difficulty, although there were no total disruptions, and (3) discontinuous, when the BM around any tumor aggregate showed total disruptions or was not seen at all.

The 7-S collagen domain of type IV collagen was purified from human kidney [24] and the fragment P1 of laminin from human placenta [25] as described previously, and antisera to these proteins were raised in rabbits. The antibodies were purified by immunoabsorption on the relevant antigen coupled to Sepharose 4B. The laminin P1 antibodies were cross-absorbed with 7-S collagen and vice versa. There was no cross-reaction between the two antibodies in the radioimmunoassay.

The sections for immunohistochemical staining were deparaffinized and treated with 0.4% pepsin (Sigma Chemical Co., St. Louis, Missouri) to enhance the availability of the antigenic determinants [26,27]. To inactivate the endogenous peroxidases the sections were exposed to a 0.1% solution of hydrogen peroxide in absolute methanol, and then stained with the peroxidase-antiperoxidase procedure [28]. Normal rabbit serum and phosphate-buffered saline were used instead of the primary antibody for control stainings.

RESULTS

All 18 basal cell tumors of the skin showed a BM containing both laminin and type IV collagen around neoplastic epithelial

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Abbreviations:
BCC: basocellular carcinoma
BM: basement membrane
BSC: basosquamous carcinoma

sheets. The staining for laminin P1 and 7-S collagen was identical in each case. The epidermal and capillary BMs stained for laminin P1 and 7-S collagen in every sample, indicating that the variations in the tumor BMs were not due to technical artifacts. The control stainings with normal rabbit serum and phosphate-buffered saline were all negative.

The BM was continuous and distinct in the 6 nonfibrosing BCCs regardless of the differentiated (3 cases) or nondifferentiated (3 cases) appearance of the epithelial component (Table II, Figs 1, 2). The most regular and distinct BM was found around those tumor islands that had a palisaded outer cell row. The 7 fibrosing BCCs possessed a BM that was discontinuous in 3 cases (all differentiated) and continuous but indistinct in 4 cases (all nondifferentiated) (Fig 3). Seven BCCs also showed staining for both laminin P1 and 7-S collagen within the tumor cell clusters.

TABLE I. *Classification of the basal cell tumors studied*

	Total	Nonfibrosing/fibrosing
Basocellular carcinomas:		
Differentiated	6	3/3
Nondifferentiated	7	3/4
Basosquamous carcinomas	5	0/5
Total	18	6/12

For classification criteria see *Materials and Methods.*

TABLE II. *Integrity of basement membrane detected by immunostaining in the basal cell tumors*

Tumor classification	Continuous and distinct BM	Continuous but indistinct BM	Discontinuous BM
Basocellular carcinomas:			
Nonfibrosing	6	0	0
Fibrosing	0	4	3
Basosquamous carcinomas	0	0	5

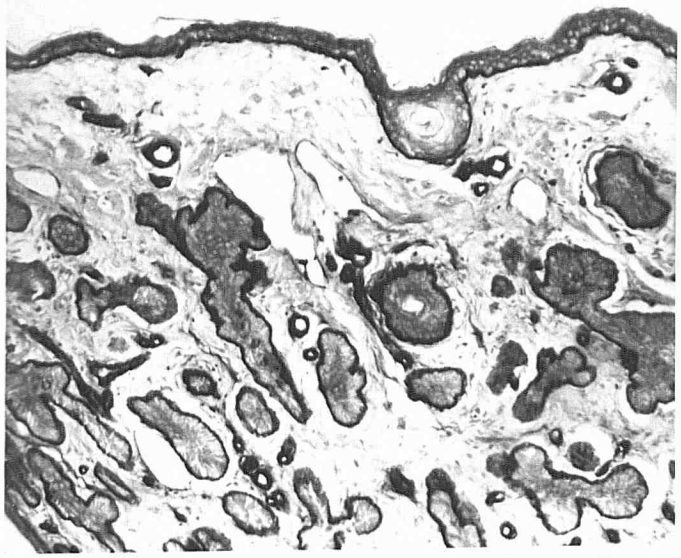

FIG 1. Immunoperoxidase staining for laminin in a nonfibrosing BCC. The basement membranes around the tumor islands and capillaries and beneath the epidermis are continuous and distinct (× 140).

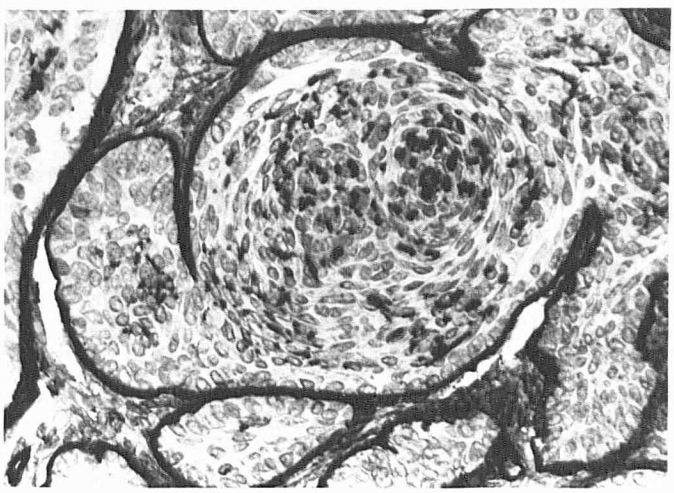

FIG 2. Immunoperoxidase staining for the 7-S collagen domain of type IV collagen in a differentiated nonfibrosing BCC. The tumor islands show adnexoid differentiation and are surrounded by a continuous, distinct BM. The tumor clusters contain fragmented BM material (× 350).

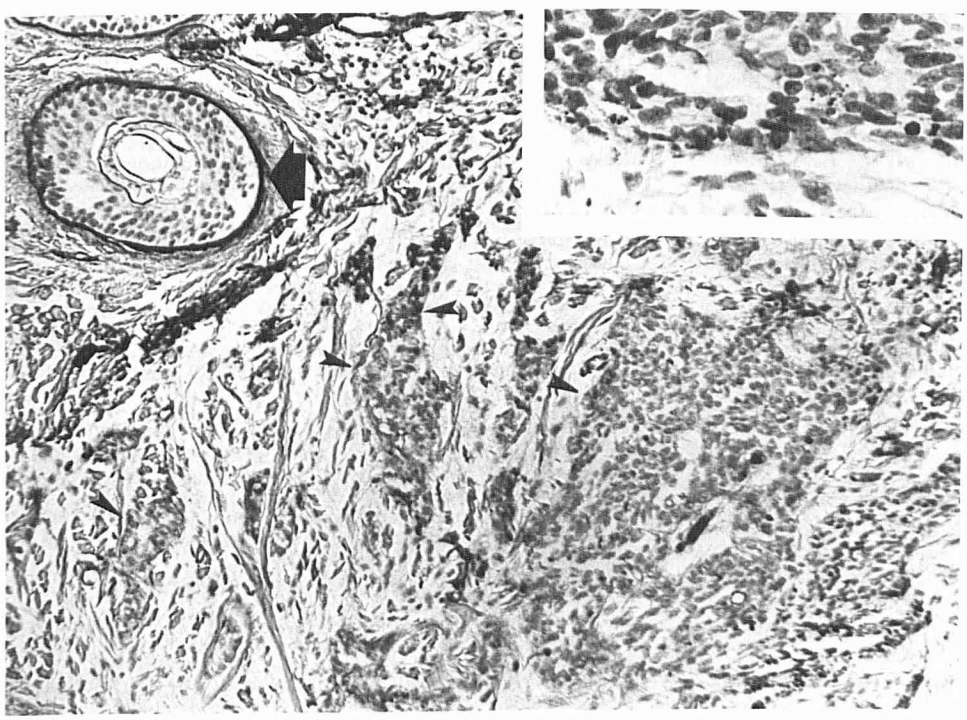

FIG 3. Immunoperoxidase staining for the 7-S collagen domain of type IV collagen in a fibrosing BCC. The normal hair follicle on the left (*arrow*) has a continuous BM, but the basocellular carcinoma sheets are devoid of BM in the fibrosing tumor area (*arrowheads*). The *inset* shows the epithelial-stromal border of the tumor at a greater magnification (× 100; *inset* × 284).

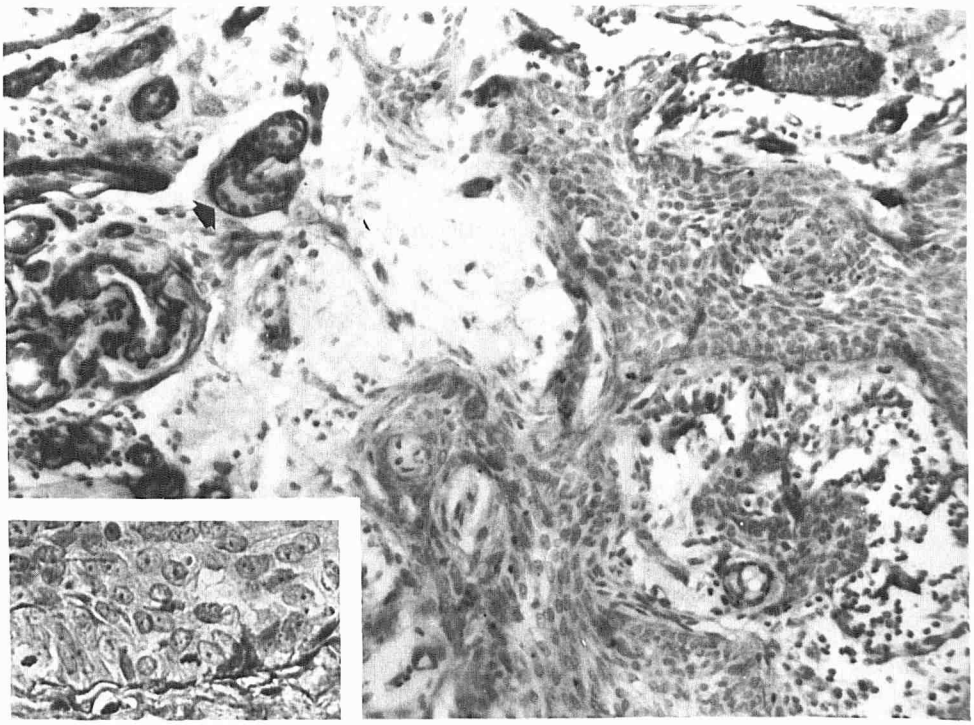

FIG 4. Immunoperoxidase staining for laminin in a BSC. The tumor sheets are devoid of BM. The *arrow* shows a normal BM around hidrous glands on the left. The connective tissue stroma is abundant and fibrosing. The *inset* shows the intermediary cell type of the tumor (× 160; *inset* × 284).

The 5 BSCs, which were all of a fibrosing type, contained a discontinuous BM. The disruptions were seen in fibrosing areas representing nonpalisading tumor cells of an intermediate type (Fig 4). Two of the cases also featured staining within the tumor cell clusters.

DISCUSSION

The demonstration of disruptions and irregularities in the BMs of fibrosing BCCs and BSCs stained with antihuman laminin P1 and antihuman 7-S collagen is a new finding, and such defects in the BM could be connected with the more aggressive nature of these tumors compared with the more common nonfibrosing BCCs [1,3].

Our finding of a continuous, distinct BM around nonfibrosing BCCs agrees with earlier studies [15,19,20,29]. Adnexoid differentiation had no influence on BM integrity in these tumors, a finding which may be explained by the fact that the outer border of the tumor cluster contained nondifferentiated basal cells even in the differentiated BCCs, this border being capable of producing BM material. The role of epidermoid differentiation of BSCs is difficult to evaluate, since all the BSCs in our material were fibrosing.

The reasons for the BM discontinuities in BCCs and BSCs are not clear. A 2-fold increase in collagenase activity compared with normal human skin has been demonstrated in BCCs, and traced to the stromal component of the tumor [30]. The activities of BM-degrading enzymes have not been studied in BCCs and BSCs as yet, but the type IV collagenase activity has been demonstrated in invasive and metastatic cases in breast carcinoma and some other tumor cell lines [31–33]. It remains to be studied whether the BM defects in BCCs and BSCs are due to analogous enzymatic degradation or to defective BM biosynthesis.

Laminin and type IV collagen were found within tumor clusters surrounded by a distinct BM, appearing focally and mostly in large tumor clusters. At the level of the light microscope it cannot be stated whether this BM material was intra- or extracellular. The occurrence of this BM material may simply reflect the fact that the tumor cells situated in the center of the cluster are also capable of synthesizing it.

Since the fibrosing types of BCC are by no means uncommon, such BM defects must also be widespread, whereas metastasizing is rare. Our results suggest that discontinuities and irregularities of the BM seem to be associated with local aggressive behavior on the part of cutaneous basal cell tumors, although such BM defects are not necessarily accompanied by a high metastatic potential for the tumor cells.

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